

# THE TOXIC PROPERTY OF SULPHUR<sup>1</sup>

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## INTRODUCTION

Since the introduction of spraying for the control of parasitic fungi there has been developed a large number of fungicidal mixtures. Some have proved effective for the control of one organism and some for another, none of them having universal fungicidal value. Because of its abundance, low cost, and its effectiveness under certain conditions, sulphur has been employed in many of these mixtures. The fact that it has toxic or fungicidal properties has been conclusively demonstrated. In this work, an attempt has been made to determine if possible the exact nature of this fungicidal property, that is, to determine or evaluate the chemical compound or compounds in which this toxic property is resident, at the same time to relate this toxic property to conditions under which it may be consistently manifest, thus warranting its general use as a fungicide.

The use of sulphur as a fungicide probably antedates that of all other substances. The chemical and physical properties of sulphur, especially its existence in so many forms, have led to its employment as a fungicide in a variety of ways. Regardless of the form in which it is employed, whether as a compound or as uncombined sulphur, there seem to be necessary certain chemical or physical changes before its toxic properties are exhibited. Toxicity has been attributed to many of the forms, for example, to such products of combined sulphur as various sulphides, thiosulphates, sulphur dioxide, sulphuric and sulphurous acids, and also to uncombined sulphur as flowers, or even as sulphur in a more finely divided state, that is, as colloidal sulphur. However, there seems to be no tangible evidence in the past work that toxic properties can be attributed directly to any one of these forms, the presence of which might thus determine its value as a fungicide. The exact state or states in which sulphur is toxic was left as a matter of considerable speculation.

<sup>1</sup> An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

<sup>2</sup> A fellowship established by the Crop Protection Institute for the investigation of sulphur as a fungicide.

## HISTORY

The generally employed sulphur sprays, namely, flowers of sulphur and the various sulphide compounds, have been only partially effective in controlling fungous diseases. It will not be necessary in this paper to go into a historical discussion of the effectiveness of these sprays, as such discussions are reported frequently by agricultural experiment stations and horticultural societies in bulletins and spray calendars and my own conception of the practical problems involved will be stated below. This work has to do largely with the fungicidal properties of sulphur.

The toxicity of the flowers of sulphur has been attributed to several compounds, of which hydrogen sulphide, sulphur dioxide, sulphurous and sulphuric acids, and volatile sulphur are more often given. Pollacci ('07) believes that sulphur is transformed into sulphuretted hydrogen, the vapors of which have a very energetic action on the fungi. This view, however, has received but little support and has been proved untenable by Bourcart ('13). He was unable to collect any of this gas on passing air from sulphur through solutions suitable for retaining the gas. Foreman ('10) could obtain no inhibition of germination with spores of *Botrytis cinerea*, using a saturated solution of sulphuretted hydrogen. Similar results were obtained by Barker, Gimmingham, and Wiltshire ('20). It is at present generally accepted that hydrogen sulphide is not a factor as a fungicidal property of sulphur.

The view that the toxic action of sulphur is due to sulphur dioxide has received considerable support. Sostegni and Mori ('90), Blodgett ('13), Butler ('17), and Kuhl ('21) conclude that the toxic property of sulphur is due to this gas. They believe that the gas is slowly produced by the oxidation of the sulphur. In the papers cited there is little substantiating experimental evidence, other than the fact that the toxic compound is volatile. Contrary views are held by Bourcart ('13) who states that "Sulphurous acid must not be dreamt of; 1/40,000 of this acid in the air would burn the leaves." In a series of experiments he could not collect any sulphur dioxide evolving from sulphur at temperatures up to 50° C. Barker, Gimmingham, and Wiltshire ('20) obtained good germination of spores of *Nectria ditissima* in a 1:100 solution of sulphur dioxide. Closed-ring experiments, however, gave limits of .005 per cent and .0005 per cent for the germination of spores of *Sclerotinia fructigena*, *Fusicladium den-*

*driticum*, *F. Pyrinum*, *Botrytis cinerea*, and *Nectria ditissima*. They conclude that sulphur dioxide cannot be a factor.

Marcille ('11) attributed the toxic property of sulphur to sulphur trioxide and sulphuric acid in the control of grape mildew. A similar conclusion was arrived at by Moissan ('04) who was able to obtain this gas from the spontaneous oxidation of different kinds of sulphur at ordinary temperatures. As far as the author is aware, these results have never been confirmed, and Bourcart ('13) and Barker, Gimmingham, and Wiltshire ('20) proved on the contrary that sulphur trioxide and sulphuric acid do not contribute to the fungicidal property of sulphur.

That sulphur is toxic because of its volatilization as such is probably the view most commonly held at the present time. The fact that spores are inhibited in germination when not in direct contact with the sulphur particle has been frequently demonstrated. Smith ('06), working with asparagus rust, concluded that sulphur acts by its fumes but that the sulphur must be uniformly distributed to be effective in controlling the disease. He found that the disease was best controlled in air pockets which aided in preventing a too rapid spreading and dilution of the fumes. Similar views are held by Mares and Mohr (see Bourcart, '13), Bioletti ('07), Bourcart ('13), Barker, Gimmingham, and Wiltshire ('20), Doran ('22), and others.

The conditions under which sulphur is volatile or under which volatile substances are formed from sulphur have been investigated by Marcille ('11), Bourcart ('13), Blodgett ('13), Kuhl ('21), and Doran ('17, '22), with the following general conclusions: (1) a certain temperature must be maintained, usually above 25° C; (2) oxygen is necessary; (3) sunlight is a possible factor; (4) the influence of the leaves and spores is considered by some a factor. These conclusions were arrived at by the use of flowers of sulphur.

The toxicity of other forms, such as finely divided sulphur and the various sulphides, has been investigated by a number of workers. Doran ('22) found that Schloesing's precipitated sulphur<sup>1</sup> was more effective in killing spores of *Venturia inaequalis* than any of the finely divided sulphurs used. Atomic sulphur<sup>2</sup> has been reported effective.

<sup>1</sup> Manufactured by Usines Schloesing Freres et Cie., of Marseille, France.

<sup>2</sup> Prepared by the General Chemical Co., New York and Baltimore.

Since the origination of lime sulphur as an orchard spray by M. F. Dusey of Fresno, California, in 1886, there has been a number of studies made on its effectiveness as a spray and on its chemical composition. The first of these of importance was by Thatcher ('06). He found that lime sulphur contained for the most part calcium polysulphides, calcium thiosulphate, and small quantities of sulphites and sulphates. Haywood ('09), using the same methods, obtained similar results. When he dried the mixture the polysulphides disappeared and increasing amounts of precipitated sulphur were formed. He attributed the fungicidal value of lime sulphur particularly to the thiosulphates and possibly to a combined or a summation of the toxic properties of all the compounds formed exclusive of sulphur.

Van Slyke, Bosworth, and Hedges ('10) made some chemical determinations of lime sulphur when the ingredients were varied. They came to the conclusion that a mixture containing a high proportion of sulphur had the largest amount of calcium pentasulphides and a greater fungicidal value. They proposed the following formula: 80 lbs. sulphur, 36 lbs. calcium oxide, and 50 gallons water. This formula is the one in general use at the present time. Their chemical determinations gave about the same results as those obtained by Haywood ('09). Ruth ('13), in a study of lime sulphur and lead arsenate mixtures, found that no arsenic sulphide was formed. The proportion of thiosulphates and sulphites was increased in this mixture, and he attributed the increased effectiveness of the spray to the presence of additional quantities of these compounds. There was no experimental evidence for this, and his chemical determinations did not show the presence of any particular toxic compound. Harris ('11) made chemical determinations of lime sulphur mixtures and found about the same amounts of sulphides, sulphites, etc., as Haywood. He also stated that filtering was unnecessary. Official methods for the determination of the compounds formed in lime sulphur are given by Roark ('20) and Winter ('20).

The above studies on lime sulphur have had to do with freshly prepared mixtures. Vermorel and Dantony ('19) gave a number of reactions that probably took place in lime sulphur mixtures and the compounds formed when the mixture was aerated. They found that the polysulphides soon disappeared after the spray was applied. The calcium thiosulphates gradually decreased, and sulphites, sulphates, and free sulphur increased. Barker,

Gimingham, and Wiltshire ('20) concluded that calcium thio-sulphate, hydrogen sulphide, and sulphur dioxide were all slightly toxic but not sufficiently so to account for the fungicidal value of lime sulphur. The calcium pentasulphides were toxic, but since they disappeared in a few hours the lasting toxicity of lime sulphur could not be attributed to them. They concluded that the lasting toxic property must be due to precipitated sulphur. Doran ('22) also found that the sulphides decomposed very rapidly, especially when dried slowly.

Several other sulphide preparations have been employed as fungicides but have proved more or less ineffective as a lasting spray because their retention on the tree as sulphides is difficult to maintain. Their caustic nature frequently results in severe burning.

In testing the toxicity of sulphur and its compounds considerable confusion has developed owing to the variation in resistance of different species of fungi. Barker, Gimingham, and Wiltshire ('20) found that germination of the spores of *Sclerotinia fructigena* and *Phragmidium subcorticium* were entirely inhibited in a suspension of flowers of sulphur in Van Tieghem cells. Germination of *Fusicladium dendriticum* and *Cladosporium fulvum* were 50 per cent inhibited, while that of *Nectria ditissima*, *Botrytis cinerea*, and *Verticillium* sp. was not at all inhibited. When the flowers of sulphur was used Doran ('22) found that a much higher temperature was necessary for the killing of spores of *Botrytis cinerea* than for *Venturia inaequalis* and a higher temperature for the latter than for spores of *Sclerotinia cinerea*.

#### EXPERIMENTAL

Since most of the evidence listed in the foregoing references points to sulphur as being the toxic agent regardless of the sulphur mixtures used, it was first thought important to study the influence of the sulphur particle and molecule on the germination of spores. The Van Tieghem cell and the hanging-drop culture method, later slightly modified, were employed. The percentage of germination of the spores was used as an indication of toxicity. The organisms used were selected from the group of strict parasites most of which are of economic importance. It was also necessary to select those that sporulated readily. The following forms were used: *Colletotrichum Gossypii*, *Sclerotinia cinerea*, *Botrytis cinerea*, *Glomerella cingulata*, *Gloeosporium*

*venetum*, *Macrosporium sarcinaeforme*, *Phomopsis Sojae*, and *Actinomyces Scabies*. These organisms were grown on potato agar prepared according to the method of Duggar, Severy, and Schmitz ('17), and spores were taken from cultures 10-15 days old.

The culture solution used in the hanging drops and in which the sulphur particles were suspended was a slightly buffered mixture containing mannite, phosphoric acid, and sodium hydroxide. The solution was prepared according to the method of Karrer and Webb ('20), as follows: Stock solutions of M/5 mannite in M/10 phosphoric acid and M/5 mannite in M/5 sodium hydroxide were made. Equal quantities of the M/5 mannite-M/10 phosphoric acid were placed in each of 10 flasks and successively increasing proportions of M/5 mannite-M/5 sodium hydroxide were added. The flasks were plugged with cotton, sterilized at 15 lbs. pressure for 15 minutes, and allowed to stand for a few hours. Titrations made by the colorimetric method (Clark, '20) showed the mixtures to have the following range of hydrogen-ion concentrations:  $P_H$  1.6, 2.4, 3.2, 4.2, 5.2, 5.8, 6.4, 6.8, 7.4, 8.4.

#### EXPERIMENT I. TOXICITY OF THE FLOWERS OF SULPHUR

Since sulphur in the form of flowers is insoluble in any solution that can be used for the growing of fungi, it was necessary to test its toxicity in the form of suspensions. Twenty test-tubes were provided with pipettes that extended through the cork stoppers and to the bottoms of the tubes. By this means drops could be transferred readily to the hanging-drop cells. These test-tubes constituted a duplicate series of 10 each, and 5 cc. of each of the slightly buffered solutions were added to the tubes so that each tube represented a particular hydrogen-ion concentration. To one series .5 gm. of flowers of sulphur was added to each tube.

The technique of the planting of the hanging-drop cultures was essentially the same as that used by Webb ('21) in his germination studies, and was as follows: Ground glass rings were cemented to glass slides by means of parawax and petrolatum. Two of these rings were placed on each slide, and 20 slides constituted a series for each organism. This gave duplicate cultures for each hydrogen-ion concentration. A few drops of the sulphur suspension to be tested for its toxicity were placed in the bottom of the two cells. Another drop was placed on a clean

sterile glass slide. A loop-full of spores was placed in this drop and the spores evenly distributed throughout the drop. By means of a small sterile glass rod a small portion of this drop was transferred to a clean sterile cover glass and the latter inverted over the glass cell. The cell was made air-tight by sealing with petrolatum. In like manner cultures were made representing each hydrogen-ion concentration both with and without sulphur. The series of hanging-drop cultures were then kept at room temperature. Examinations were made at the end of 16 and 24 hours.

After examining some of the preliminary cultures it was found that considerable irregularity in germination existed. Some types of spores would remain on the surface of the drops and often would not be in close proximity to the sulphur. Other types of spores were found to be in the center of the drops with the sulphur particles. Different-sized drops would often result in irregular germination in the control cultures. With some organisms the number of spores in the drop influenced the rate of germination. To eliminate such chance for error a definite spore suspension was made and the drop on the cover glass was spread over a much larger surface, giving it more the nature of a smear. In this way a more even distribution of both the spores and the sulphur particles was obtained. The results of the experiment are recorded in table I and figs. 1-4.

Sulphur in this form was found to be directly toxic to only two of the organisms used. In the other forms the spores were not only not inhibited from germinating but the germ tubes grew normally when in direct contact with the sulphur particles. It can only be concluded from these results that if the flowers of sulphur has a general fungicidal value it must be due to some change in the form of sulphur and that this change takes place under different conditions than were obtained in closed-ring Van Tieghem cells. Within the usual range for germination the hydrogen-ion concentration influenced the results but slightly.

#### EXPERIMENT 2. FINELY GROUND FLOWERS OF SULPHUR

Since the ordinary flowers of sulphur was toxic to two of the organisms, it was concluded that there was a toxic property present but in a very dilute form. If physical conditions influenced the production of this property it was thought that possibly a finely ground product might be more effective. To obtain sulphur in this state an electrically driven excentric mortar, as used for

crushing yeast cells, was employed. Eight gms. of flowers of sulphur were mixed with 3 gms. of diatomaceous earth (Kieselguhr), and the mixture ground for 14 hours. One-half gm. of this mixture was added to each test-tube containing the slightly buffered solution of the different hydrogen-ion concentrations and the toxicity determined as before. An attempt was made to grind the sulphur without the diatomaceous earth but the sulphur had a tendency to cake and did not grind well. Other substances are being tried with the hope of eliminating diatomaceous earth. Results of this experiment are given in table I, figs. 1-4.

Sulphur in this state was found to be more toxic than the flowers of sulphur unground. A more marked influence of the hydrogen-ion concentration was noted, the range showing the greatest toxicity being between  $P_H$  4.2 and 5.4. The increased toxicity at this point is attributed to one of 2 possibilities: first, the spores may be less resistant at this point, or second, the toxic form or conditions of sulphur may have been produced in greater amounts at this range. At any rate the hydrogen-ion concentration and the fineness of the particle contributed to the increased toxicity of the sulphur. The fineness of the particle did not seem to be the direct cause, as germ tubes grew normally after the initial retardation, even though they were directly in contact with the sulphur particles.

### EXPERIMENT 3. COLLOIDAL SULPHUR

Sulphur readily assumes the colloidal state. The element sulphur has been known since the beginning of history, and records show that colloidal sulphur was prepared and studied as early as the seventeenth century. "Lac Sulfurus," a colloidal form of sulphur, was prepared in 1765 by Stahl (1766) and was used at that time for medicinal purposes. Fourcroy (1790), Berthollet (1798), Berzelius (1808), and Magnus (1827) were early contributors to the study of colloidal sulphur. Present-day methods for the preparation of colloidal sulphur are found in papers by Svedberg ('09), Himmelbauer ('09), Raffo ('08, '11), Odén ('13), v. Weimarn and Molyschew ('11), Kelber ('12), and others.

Colloidal sulphur exists in two forms, depending upon the degree of hydration. The form having a very high degree of hydration will be discussed in this paper as the hydrophilic colloidal sulphur and is identical with the product prepared by Raffo and Mancini ('11) and Odén ('13) and called "soluble



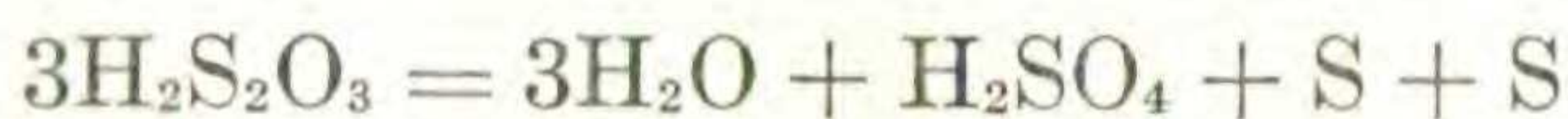
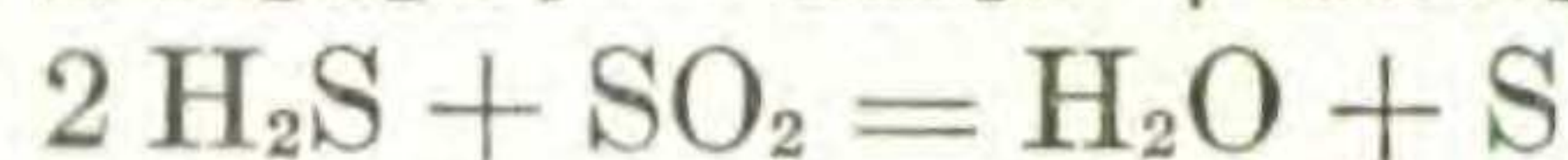
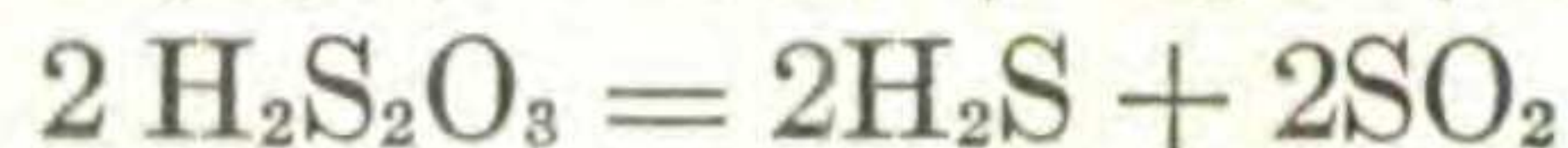
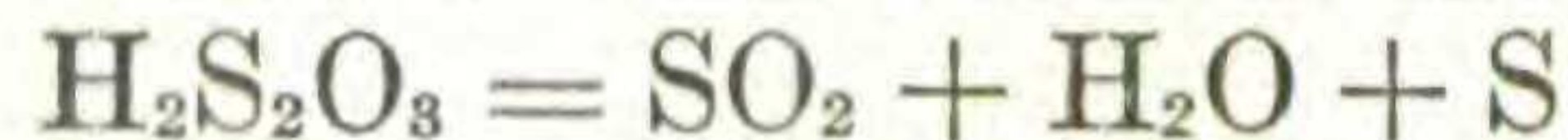
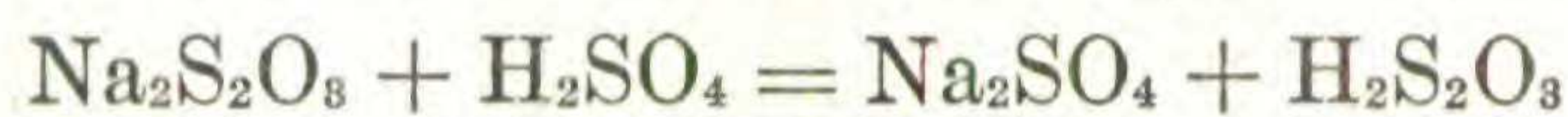
colloidal sulphur." The other form of colloidal sulphur is that first prepared by v. Weimarn and Molyschew ('11). This last has a very low degree of hydration and will be designated in this paper as hydrophobic colloidal sulphur. A more detailed description of these forms will be given in a subsequent section.

The hydrophilic colloidal sulphur was prepared according to the methods of Raffo and Mancini ('11) and Odén ('13) with certain modifications. Fifty gms. of pure crystalline sodium thio-sulphate were dissolved in 30 cc. of distilled water; 70 gms. of concentrated sulphuric acid, sp. gr. 1.84, arsenic free, were weighed into a glass cylinder of 300 cc. capacity. The cylinder was placed in a vessel of cold water and the saturated solution of sodium thiosulphate added very slowly with occasional stirring. The mixture was then allowed to cool and 30 cc. of distilled water added. It was then placed on the water bath and warmed at 80° C. for 10 minutes, and filtered through glass wool to remove insoluble sulphur. The filtrate was cooled and allowed to stand for 12 hours. It was again warmed, filtered through glass wool, and the filtrate cooled. This warming, filtering, and cooling was repeated until no more insoluble sulphur came down. The final filtrate was a slightly turbid yellowish solution. This was centrifuged for 30 minutes at 1500 revolutions per minute. A portion of the colloidal sulphur was thrown out of solution. The supernatant liquid was a clear yellowish solution and was saved for further purification. The residue was washed in cold distilled water and again centrifuged for the same length of time and at the same speed. The supernatant liquid was again yellowish and was saved. The washing and centrifuging of the residual colloidal sulphur were repeated until the residue peptized in water gave a reaction of  $P_H$  4.2. This colloidal suspension was faintly yellow and upon standing 1 week some of the particles settled out, the solution retaining its faint yellow color. Upon drying and weighing, the suspension gave a percentage of sulphur of 3.4.

The supernatant liquids collected from the above were treated with a concentrated solution of sodium chloride, whereby the yellowish colloidal sulphur was coagulated. The sodium chloride was added until no more coagulum seemed to form. The coagulum was easily centrifuged out and re-peptized in 10 cc. of distilled water. The color of this solution was a deeper yellow and only very slightly turbid. This colloidal suspension gave a reaction

of  $P_H$  4.2 and did not settle out on standing for 2 months. On drying and weighing, the solution was found to contain 1.6 per cent sulphur. This latter preparation was a typical hydrophilic colloidal sulphur and was more nearly a true "soluble" sulphur than the product obtained from the method of Raffo and Mancini ('11). The first preparation was a mixture of hydrophilic and hydrophobic colloidal sulphur. Odén ('13), in a detailed study of this type of colloidal sulphur mixtures, found them to contain particles of different sizes ranging from the molecular to particles easily discernible under the low power of the microscope. He was able to obtain suspensions with particles varying from the smallest to the largest by fractional coagulation with sodium chloride. Particles of larger size were more easily coagulated than the smaller ones. In colloidal sulphur suspensions of this kind the particles have a tendency to collect themselves into groups, forming larger particles which settle out rapidly. The smaller the particles the slower this takes place and in hydrophilic colloidal sulphur suspensions only a small amount of settling out can be noted after several months.

The chemical reactions involved in the formation of colloidal sulphur prepared by this method is given by Odén as follows:



Further chemical reactions will be given in a subsequent section of this paper.

The method for the preparation of hydrophilic colloidal sulphur was later varied in accordance with the method used by Freundlich and Scholz ('22). After the filtration through glass wool concentrated sodium chloride was added and the mixture centrifuged. The coagulum was then peptized with 100 cc. of distilled water and the insoluble sulphur centrifuged out. The peptized sulphur solution was treated 3 times with 25 cc. of saturated sodium chloride and finally peptized in 100 cc. of distilled water.

Another method for the preparation of hydrophilic colloidal sulphur was that first used by Selmi ('52) and was as follows. Sulphur dioxide was passed into distilled water until a saturated

solution was formed. Hydrogen sulphide was then passed into the sulphurous acid solution, care being taken not to have an excess of the hydrogen sulphide, as it precipitates the hydrophilic colloidal sulphur forming the hydrophobic colloid. The solution was then centrifuged to remove the larger particles and the supernatant liquid coagulated with sodium chloride. The coagulum was then peptized in water as before.

The hydrophobic colloidal sulphur can be prepared in a number of ways. It is the "milk of sulphur" formed when sulphur is precipitated out of solution. It was prepared in this work by the method used by v. Weimarn and Molyschew ('11) which was as follows: Sulphur was recrystallized in toluol and the toluol evaporated off at 60–70° C. Five-tenths gm. of this was heated with 125 cc. of fresh distilled absolute alcohol in a reflux condenser for 60 minutes. Seven cc. of this hot solution were poured into 293 cc. of distilled water at room temperature. The suspension prepared in this way was white and turbid. This was centrifuged and resuspended in water. The sulphur particles settled out of this suspension in a comparatively short time.

In determining the toxicity of these forms of colloidal sulphur the same method was used as in the preceding tests. With the hydrophilic colloidal sulphur, however, it was necessary to make a much weaker suspension. The stock colloidal suspensions contained about 1.5 per cent sulphur. Five cc. of this stock suspension were diluted to 25 cc. with distilled water, and then 1 cc. of this was added to each of the hydrogen-ion concentrations. This gave a further dilution of 1:5 and resulted in a very weak suspension of colloidal sulphur. After a preliminary test, however, the hydrophobic colloidal sulphurs were not diluted with water, and 1 cc. of the stock suspension was added directly to the culture solutions. The organisms used and the results are recorded in table I and figs. 1–4.

With the 6 organisms used in this experiment hydrophilic colloidal sulphur was found to be extremely toxic in the very dilute suspensions used. Only 2 of the organisms, namely, *Botrytis cinerea* and *Macrosporium sarcinaeforme*, showed a slight resistance to this suspension. In stronger suspensions germination was entirely inhibited with all the organisms used. On the other hand, hydrophobic colloidal sulphur was only slightly toxic and comparable to ground flowers of sulphur. The results indicate that sulphur is most toxic in a very finely divided state

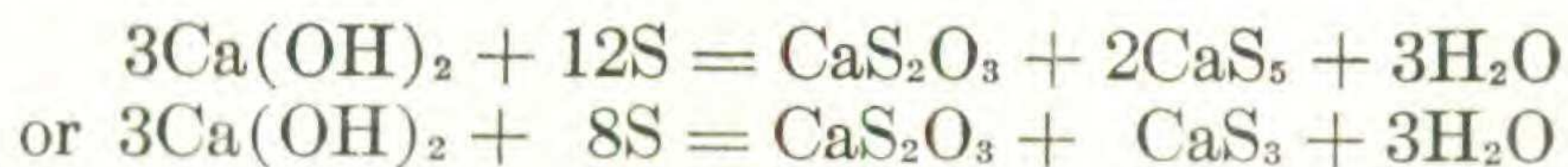
such as is found in the hydrophilic colloidal sulphur. The influence of the hydrogen-ion concentration was very striking, especially with this latter form of sulphur. Upon examination of the culture tubes containing the hydrophilic colloidal sulphur it was found that settling out was rapidly increased as the  $P_H$  increased beyond  $P_H$  5.4.



Fig. 1. Germination of spores of *Botrytis cinerea* in hanging-drop cultures: toxic action of flowers of sulphur ————; of hydrophobic colloidal sulphur ————; of hydrophilic colloidal sulphur .....; check, without sulphur ———.

#### EXPERIMENT 4. THE TOXICITY OF LIME SULPHUR

The compounds formed in lime sulphur mixtures have been fairly well determined by Haywood, Van Slyke, and others. The reactions that take place when sulphur and calcium oxide are boiled together are about as follows:



These reactions are influenced, of course, by the initial ratio of the ingredients. Varying amounts of  $\text{CaS}_3$ ,  $\text{CaS}_4$ ,  $\text{CaS}_5$ ,  $\text{CaS}_2\text{O}_3$  and  $\text{CaSO}_3$  are formed depending upon this ratio. When lime sulphur is prepared according to the Van Slyke method, that is,

boiling together 80 lbs. of sulphur, 36 lbs. of lime, and 50 gallons of water, the first reaction is the more probable one. When prepared in this way the mixture has about the following composition: sulphur as sulphides (largely pentasulphides), 80.7 per cent, as thiosulphates, 19 per cent, as sulphites and sulphates, 0.03 per cent.

Lime sulphur mixtures are extremely alkaline and their initial efficiency as a fungicide may be due partly to this causticity, that is, to the free hydroxyl ions. An experiment was performed to determine how long this causticity remained when the spray

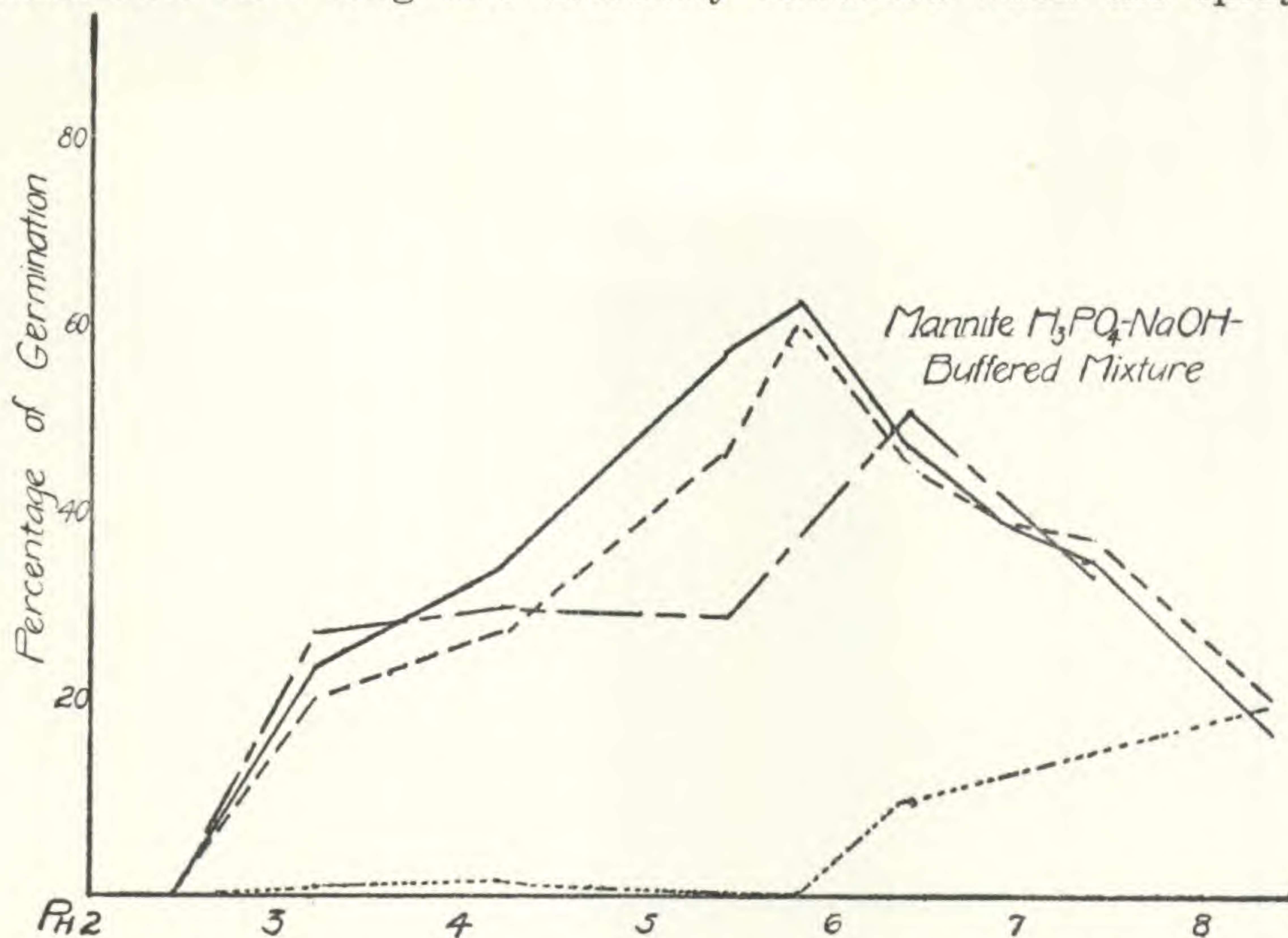


Fig. 2. Germination of spores of *Colletotrichum Gossypii* in hanging-drop cultures: toxic action of flowers of sulphur — — — —; of hydrophobic colloidal sulphur — — — — —; of hydrophilic colloidal sulphur ·········; check, without sulphur —————.

was applied, and to ascertain, if possible, whether this factor was the principal one in giving lime sulphur its prolonged effectiveness as a fungicide. For this purpose lime sulphur was prepared according to the formula given by Van Slyke, and 1 part of the lime sulphur diluted with 6 parts of water. This is a little stronger than the concentration used as a dormant spray. Twelve large moist chambers were sprayed with this mixture and kept under the following conditions: Four were exposed to dry air; a second set of 4 was placed under slightly humid conditions, and a third set of 4 in a saturated condition. After 2 hours

the lime sulphur was washed from 1 of the exposed glass dishes and the reaction determined. It was found to have changed from an initial reaction of beyond the alkaline  $P_H$  range of indicators available ( $P_H$  10.0) to  $P_H$  6.4. Likewise, at the end of the same length of time the mixture was washed from one of the vessels in the second set and tested for its reaction. The reaction in this case remained beyond  $P_H$  10.

At the end of 6 hours the reactions were again determined. The wash from the first set remained the same. In the second set the reaction had changed to  $P_H$  7.4 and in the third set it was still beyond  $P_H$  10. At the end of 24 hours a third set of readings

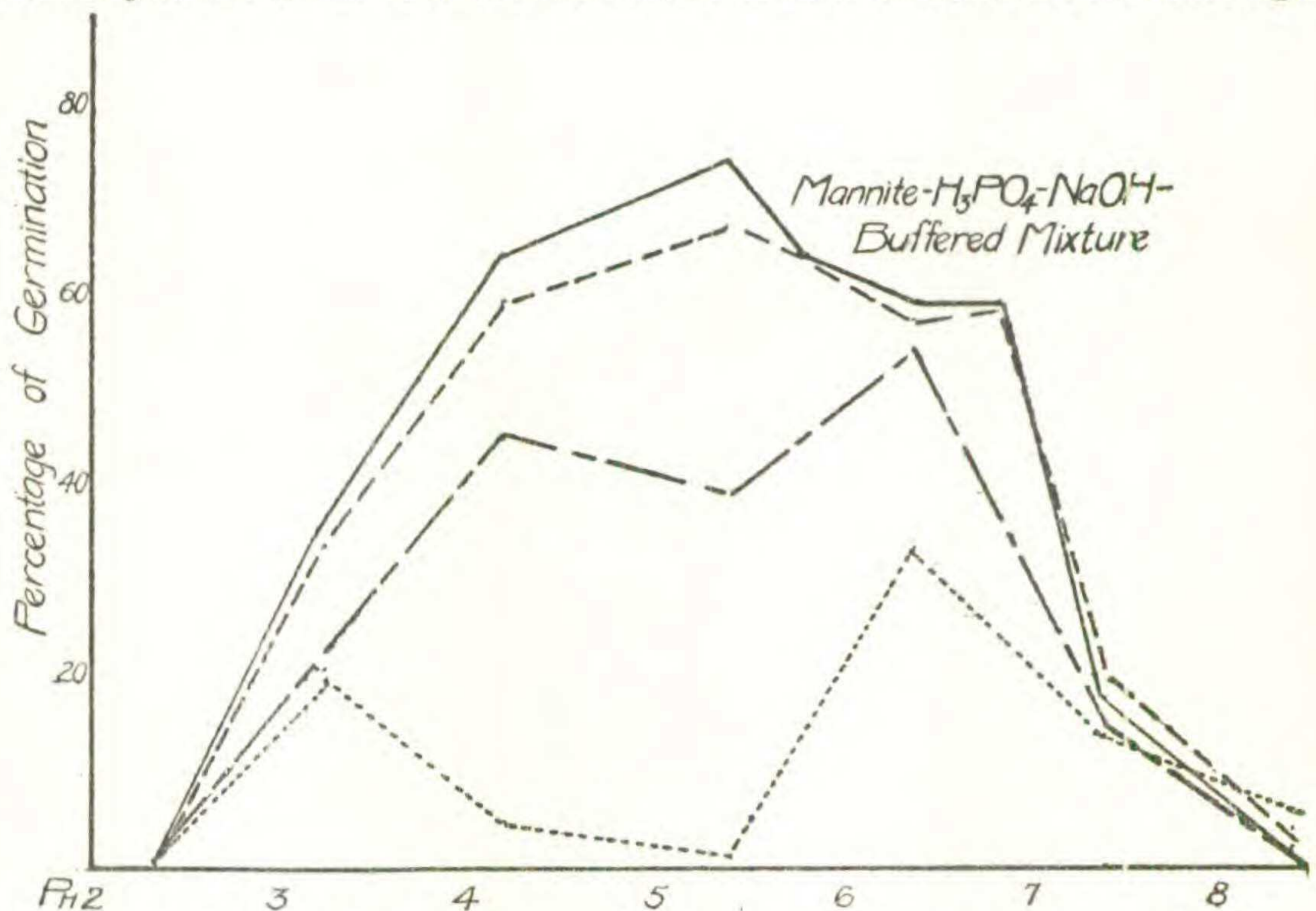


Fig. 3. Germination of spores of *Gloeosporium venetum* in hanging-drop cultures: toxic action of flowers of sulphur— — — — —; of hydrophobic colloidal sulphur — — — — —; of hydrophilic colloidal sulphur .....; check, without sulphur — — — — —.

was made. All gave the same reaction,  $P_H$  6.4. The mixture placed under the third condition did not dry out, but changed in reaction to the same point as the others. It would appear from these results that the lasting action of lime sulphur is not due to its causticity.

At this point it was thought advisable to make some chemical determinations of the exposed or changed lime sulphur. Using the same method as that listed by the Association of Official Agricultural Chemists ('20) it was found that polysulphides were absent. The percentage of thiosulphates as determined by the

Shaffer and Hartman method ('21) was 1.4. Sulphides were found to be approximately 0.1 per cent. Precipitated sulphur as determined by the carbon bisulphide method gave a percentage of 2.8. We have present then in the changed lime sulphur, precipitated sulphur, calcium thiosulphate, calcium sulphite, and calcium sulphate.

The toxicity of these individual compounds was next determined. Fifty cc. of 1:6 lime sulphur solution were set aside in a large open vessel. After 36 hours the reaction had changed to  $P_H$  6.4. The solution was then removed and the vessel washed with

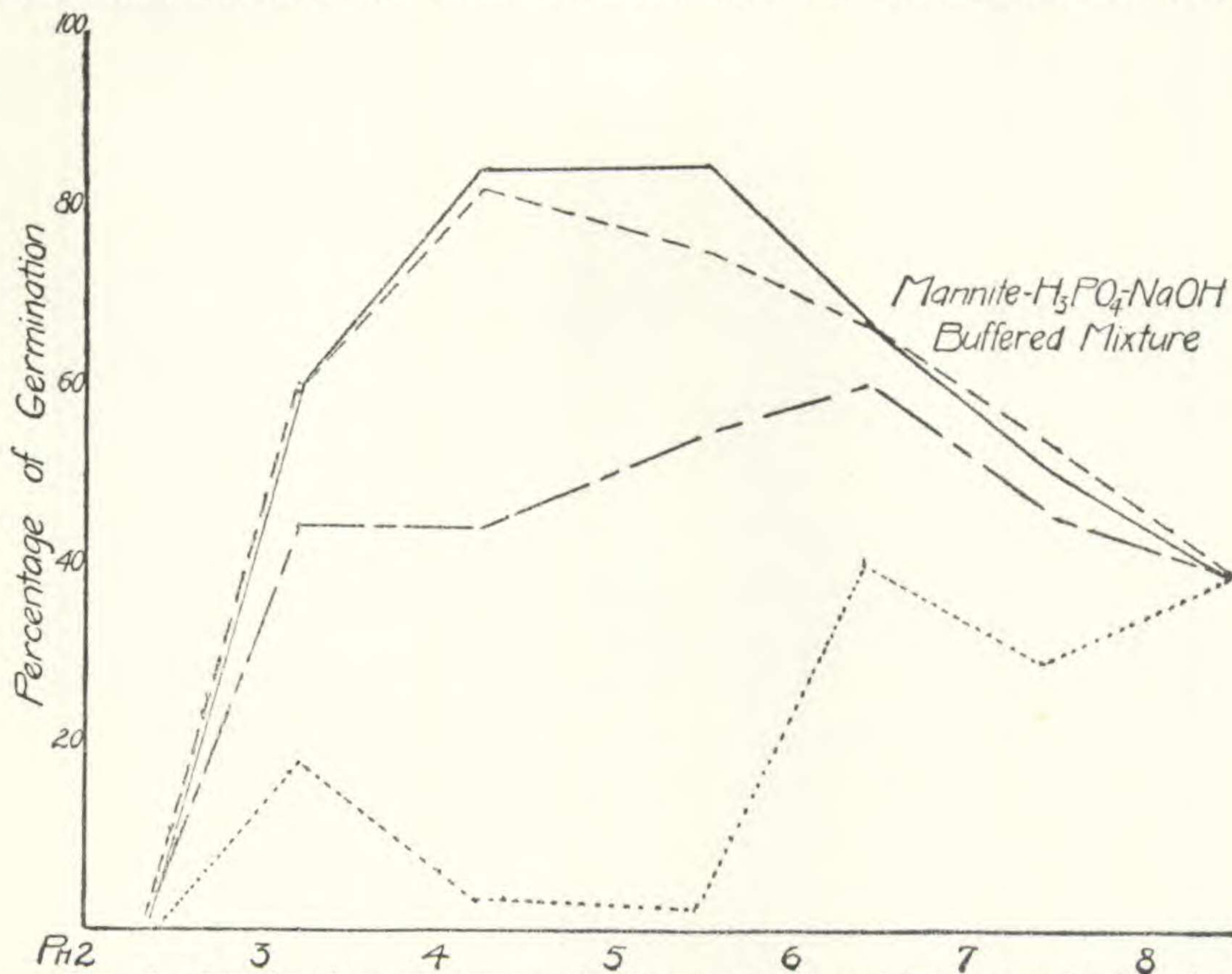


Fig. 4. Germination of spores of *Macrosporium sarcinaeforme* in hanging-drop cultures: toxic action of flowers of sulphur ---; of hydrophobic colloidal sulphur - - -; of hydrophilic colloidal sulphur .....; check, without sulphur —.

sufficient distilled water to make the total quantity up to the original 50 cc. This mixture was then centrifuged until the supernatant liquid was clear. A test was then made for calcium thiosulphate in the supernatant liquid. The percentage was 1.4. The part thrown out of the solution by the centrifuge was again washed in cold water and again centrifuged. The washing removed any soluble compound that might have been present. This washed substance was then suspended in 50 cc. of distilled water. The compounds contained in this suspension were the insoluble

compounds that were formed in the changed lime sulphur, such as the sulphites and sulphates and the precipitated sulphur. The toxicity of these compounds was determined in the same way as in the preceding experiments.

The calcium thiosulphate solution did not inhibit germination of any of the organisms used. Similar results were obtained by Armstrong ('21) in his studies on sulphur nutrition of the fungi. Accordingly, calcium thiosulphate cannot be a factor, even at this high concentration, in the fungicidal value of lime sulphur.

One cc. of the precipitated sulphur suspension was placed in each of the tubes containing the slightly buffered solution. This made a further dilution of 1:5, making this final suspension equal to that of the original changed lime sulphur, that is, 1:6. The toxicity was determined in the same way as in the preceding tests. The results are given in table I.

The results were very similar to those obtained with colloidal sulphur. The hydrogen-ion concentration influenced the toxicity in the same general way. To make sure that this toxicity was not due to the sulphites and sulphates the sulphur was coagulated out as in the case of colloidal sulphur and the toxicity again determined. The results were the same. A further test was made, using a 0.1 per cent solution of calcium sulphite, but no toxicity resulted. An attempt was next made to try to further purify the sulphur suspension by fractional centrifugation, in which the centrifuge was run very slowly, thus throwing out the sulphur and not the insoluble calcium sulphites. By repeating the centrifuging 5 or 6 times a sulphur suspension was obtained which when dried was completely soluble in carbon bisulphide. The results with reference to toxicity were the same as those cited above.

It must be concluded from these results that the lasting fungicidal value of lime sulphur is due almost entirely to the precipitated sulphur, directly or indirectly, and not to the calcium thiosulphate and the insoluble sulphites. The precipitated sulphur formed in the changed lime sulphur is not in as finely divided state as the soluble colloidal sulphur prepared by the above methods, as was shown by the slow speed with which it could be thrown out of suspension. However, its toxicity was slightly greater than that of the hydrophobic colloidal sulphur in the same concentration.



TABLE I

PERCENTAGE OF GERMINATION\*

Organism	Form of Sulphur	Hydrogen-ion concentration (P <sub>H</sub> )									
		1.6	2.4	3.2	4.2	5.4	5.8	6.4	6.8	7.4	8.4
<i>Botrytis cinerea</i> .....	Without sulphur.....	4	76	94	90	86	76	56	34	24	0
	Flowers of sulphur.....	4	74	91	91	84	68	51	33	23	0
	Ground flowers of sulphur	4	66	78	49	68	—	52	—	16	0
	Hydrophilic colloidal sulphur.....	6	60	45	10	8	—	58	—	23	0
	Hydrophobic colloidal sulphur.....	4	74	81	51	64	—	74	—	21	—
	Precipitated lime sulphur	—	60	52	20	16	—	17	22	18	—
<i>Colletotrichum Gossypii</i> .....	Without sulphur.....	0	0	24	35	57	64	48	40	36	18
	Flowers of sulphur.....	0	0	21	28	47	61	46	40	38	21
	Ground flowers of sulphur	0	0	23	25	28	—	35	—	30	—
	Hydrophilic colloidal sulphur.....	0	0	1	1	0	0	10	—	15	20
	Hydrophobic colloidal sulphur.....	0	0	28	31	30	—	52	—	34	—
	Precipitated lime sulphur	0	0	12	13	11	—	26	—	28	—
<i>Sclerotinia cinerea</i> .....	Without sulphur.....	0	48	72	94	82	80	76	29	3	0
	Flowers of sulphur.....	0	0	0	0	0	0	0	0	0	0
	Hydrophilic colloidal sulphur.....	0	0	0	0	0	0	0	0	0	0
<i>Gloeosporium venetum</i> .....	Without sulphur.....	0	4	36	65	75	65	60	60	18	2
	Flowers of sulphur.....	0	3	34	60	68	65	58	59	21	4
	Ground flowers of sulphur	0	3	21	46	40	—	55	—	15	3
	Hydrophilic colloidal sulphur.....	0	4	20	5	1	—	34	—	14	6
<i>Macrosporium sarcinaeforme</i> .....	Without sulphur.....	0	4	60	85	86	—	68	—	52	40
	Flowers of sulphur.....	0	4	61	83	76	—	68	—	55	41
	Ground flowers of sulphur	0	4	45	45	56	—	61	—	48	40
	Hydrophilic colloidal sulphur.....	0	0	19	3	2	—	41	—	30	38
<i>Phomopsis Sojae</i> .....	Without sulphur.....	0	0	1	42	31	83	—	63	37	26
	Flowers of sulphur.....	0	0	0	0	0	0	0	0	0	0

\* Average of 6-12 replications conducted at 3 different times.

## EXPERIMENT 5. THE TOXICITY OF THE VOLATILE PRODUCTS OF SULPHUR

The results of the foregoing experiments indicate that sulphur is most toxic when it is in a finely divided state, this toxicity increasing in proportion to the fineness of the particle, hydrophilic colloidal sulphur exhibiting the highest degree of toxicity. The prevailing supposition that sulphur is only toxic when in a volatile state might be justified by the assumption that finely di-

vided sulphur yields a volatile product more readily. On the other hand, the peculiar relation of this toxicity to a definite range of hydrogen-ion concentration points rather towards the probability that sulphur is toxic because of a compound produced that may be volatile, the production of this compound being affected directly by the reaction. It does not seem probable that the colloidal sulphur particle as such could be rendered non-toxic by so slight a change in reaction as has been shown to govern its fungicidal property.

To determine these points a series of experiments was arranged, using flowers of sulphur, hydrophilic and hydrophobic<sup>1</sup> colloidal sulphur. The organisms used were *Botrytis cinerea*, *Colletotrichum Gossypii*, and *Sclerotinia cinerea*. The method of procedure was the same as in the preceding experiments, with the following modifications: The spores were placed in drops of the slightly buffered solution without sulphur. The sulphur suspensions were placed only at the bottom of the cells. In this way the spores were not in direct contact with the sulphur, the distance between culture drop and cell liquid being the height of the cell, which was 8 mm. The cultures were incubated at 22° C. The results are given in table II.

TABLE II  
PERCENTAGE OF GERMINATION

Organism	Form of Sulphur	Hydrogen-ion concentration (P <sub>H</sub> )									
		1.6	2.4	3.2	4.2	5.4	5.8	6.4	6.8	7.4	8.4
<i>Botrytis cinerea</i> .....	Without sulphur.....	4	76	94	90	86	76	56	31	10	0
	Flowers of sulphur.....	4	74	91	91	84	78	51	33	—	—
	Hydrophilic colloidal sulphur.....	3	60	58	12	8	54	61	50	—	—
	Hydrophobic colloidal sulphur.....	4	71	95	84	90	74	52	32	—	—
<i>Colletotrichum Gossypii</i> .....	Without sulphur.....	0	0	24	38	53	55	58	46	—	—
	Flowers of sulphur.....	0	0	22	37	51	50	59	45	—	—
	Hydrophilic colloidal sulphur.....	0	0	10	0	0	21	43	47	—	—
	Hydrophobic colloidal sulphur.....	0	0	26	28	44	53	61	42	—	—
<i>Sclerotinia cinerea</i> ..	Without sulphur.....	0	51	84	95	82	70	57	9	3	0
	Flowers of sulphur.....	0	50	66	70	74	65	58	11	0	0
	Hydrophilic colloidal sulphur.....	0	0	0	0	0	3	24	14	2	0
	Hydrophobic colloidal sulphur.....	0	36	19	8	10	65	59	11	—	—

<sup>1</sup> Precipitated sulphur.

The results in this table are very similar to those recorded in table 1 except that the flowers of sulphur exhibited no toxic action even to *Sclerotinia cinerea* and the hydrophobic colloidal sulphur was only slightly toxic with *Botrytis cinerea* and *Colletotrichum Gossypii*. The hydrophilic colloidal sulphur exhibited the usual degree of toxicity, regardless of the fact that it was a considerable distance from the spore. Toxicity was greatest in all cases at  $P_H$  4.0–5.5, as in the previous tests.

Having determined that the toxic substance is volatile, it was thought necessary at this point to eliminate, if possible, hydrogen sulphide, sulphur dioxide, and sulphur trioxide, as factors. For these tests *Sclerotinia cinerea* was selected because it has proved to be quite sensitive to the toxic action of sulphur. Spores were placed over a saturated solution of hydrogen sulphide in a Van Tieghem cell and the cultures were incubated at 22° C. for 24 hours. Germination was not inhibited. The experiment was repeated with *Colletotrichum Gossypii* and *Botrytis cinerea* with similar results.

No toxicity could be noted with sulphur dioxide in a concentration sufficient to kill when converted into hydrophilic colloidal sulphur by the addition of hydrogen sulphide.

Sulphuric acid inhibited growth only because of its acidity, accordingly, in proportion to acidity. Positive tests for sulphur dioxide and trioxide could not be obtained in aerated sulphur suspensions that were toxic to *Sclerotinia cinerea*. These compounds, therefore, do not contribute to the toxic properties of sulphur.

#### EXPERIMENT 6. THE INFLUENCE OF $O_2$ ON THE TOXICITY OF SULPHUR

In all of the foregoing tests the only oxygen available was that present in the air enclosed in the closed-ring cells. An experiment was conducted to determine the effect of oxygen on increasing the toxicity of flowers and precipitated sulphur. Finely ground flowers of sulphur and hydrophobic colloidal sulphur were placed in the slightly buffered mixtures in the same concentration as in Experiments 1 and 2. The Van Tieghem cells were placed in Petri dishes, the bottoms of which were lined with filter-paper in which holes somewhat larger than the glass rings were cut, so that the cells might rest on the bottoms of the Petri dishes. A large drop of the sulphur suspension was placed at

the bottom of the ring. The filter-paper was saturated with water. Spores of *Sclerotinia cinerea* were placed in a drop of the culture medium without sulphur, on a cover slip which was inverted over the cell. The cells were not sealed at the top or bottom.

In the same Petri dish sealed cells were prepared. This was done for each hydrogen-ion concentration. The Petri dishes containing the cultures were arranged in a moist chamber through which air was passed. This experiment was conducted at room temperature and the percentage of germination noted after 18 hours. The results are given in table III.

TABLE III  
PERCENTAGE OF GERMINATION\*  
SCLEROTINIA CINEREA

P <sub>H</sub>	Ground flowers of sulphur		Hydrophobic colloidal sulphur	
	- O <sub>2</sub>	+ O <sub>2</sub>	- O <sub>2</sub>	+ O <sub>2</sub>
2.4	40	38	34	24
3.2	64	49	17	10
4.2	68	31	8	0
5.4	65	24	11	0
5.8	62	46	54	32
6.4	58	49	48	44

\*Average of triplicate cultures.

Another experiment was conducted, in which a weak suspension of hydrophobic colloidal sulphur which did not inhibit the germination of spores of *Colletotrichum Gossypii* at any hydrogen-ion concentration was aerated for 24 hours. Air from which the oxygen was removed with pyrogallol<sup>1</sup> was passed through a duplicate series. The toxicity was determined with spores of *C. Gossypii* in closed-ring cells in the same manner as in Experiment I. Likewise, a similar series was arranged, using an aerated suspension of flowers of sulphur. The cultures were incubated at 22° C. and the percentage of spore germination determined after 18 hours. The results are recorded in table IV.

The results of these tests prove conclusively that the toxic property of sulphur is due to an oxidation product and that finely divided sulphur is more readily oxidized at ordinary temperatures than the ordinary sublimed sulphur.

<sup>1</sup> One part pyrogallol, 5 parts NaOH, and 30 parts H<sub>2</sub>O.

TABLE IV

PERCENTAGE OF GERMINATION  
COLLETOTRICHUM GOSSYPII

P <sub>H</sub>	Hydrophobic colloidal sulphur		Flowers of sulphur	
	- O <sub>2</sub>	+ O <sub>2</sub>	- O <sub>2</sub>	+ O <sub>2</sub>
2.4	0	0	0	0
3.2	26	18	22	18
4.2	42	2	51	16
5.4	56	13	60	10
5.8	60	37	68	43
6.4	66	62	54	56

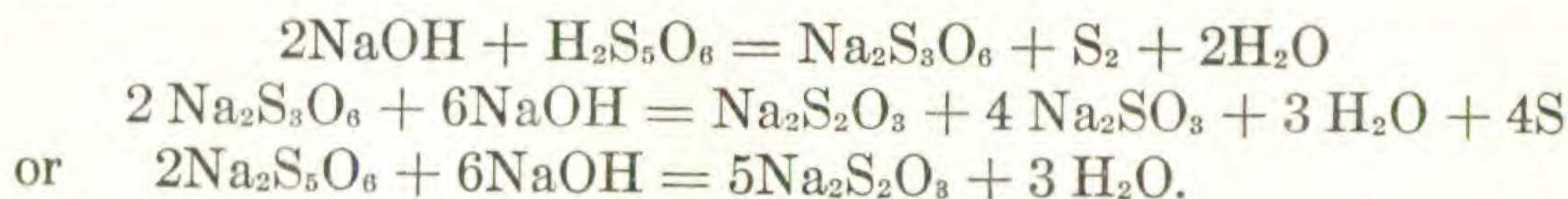
EXPERIMENT 7. THE INFLUENCE OF H<sub>2</sub>O ON THE TOXICITY  
OF SULPHUR

The influence of water on this volatile compound was next studied. Dry colloidal sulphur was prepared by centrifuging hydrophobic colloidal sulphur and the residue dried at room temperature. This was placed in the bottom of Van Tieghem cells. Spores of *Sclerotinia cinerea* were placed in sterile distilled water on sterile cover slips and inverted over the cell. The cell was not made air-tight, thereby not eliminating any other factor except water. Checks were arranged in which a suspension was used instead of the dry sulphur, other conditions being the same. All the cultures were placed in a moist chamber at room temperature. There resulted from this experiment no inhibition when dry sulphur was used, while the suspension gave the same amount of inhibition as reported in table III. Oxygen and water are necessary factors in the formation of the toxic volatile compound of sulphur.

CHEMISTRY OF HYDROPHILIC COLLOIDAL SULPHUR

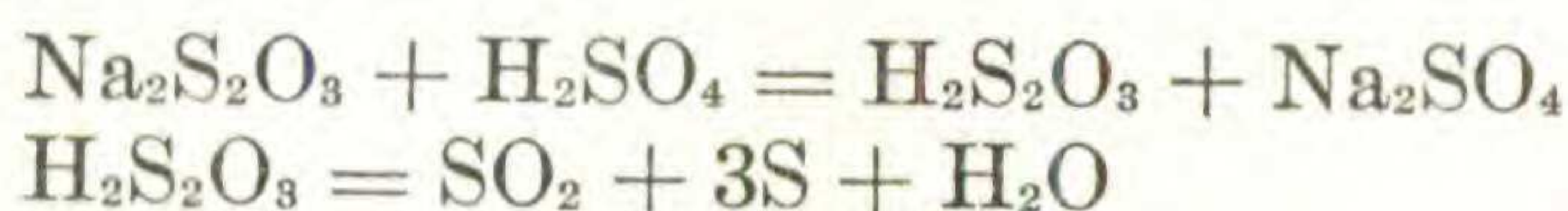
The results of all the foregoing experiments point towards hydrophilic colloidal sulphur as containing the toxic substance produced by the oxidation of the ordinary forms of sublimed and precipitated sulphur. It is as toxic in closed-ring cells where little oxygen is available as in open aerated cells. The other forms of sulphur tried are toxic only when oxygen is present. Hydrophilic colloidal sulphur is toxic at 21–22° C. to *Botrytis cinerea*, *Macrosporium sarcinaeforme*, *Gloeosporium venetum*, and *Colletotrichum Gossypii*, all of which are very re-

sistant and grow normally in a suspension of flowers of sulphur at temperatures below 25° C. Because of these facts it is logical to assume that the toxic property of sulphur is due to a compound formed by the oxidation of sulphur. Having eliminated the more common oxides and acids of sulphur it was thought that this toxic compound might be one or a mixture of the more complex polythionic acids. At any rate hydrophilic colloidal sulphur contains such an acid. The chemistry of hydrophilic colloidal sulphur has been studied by a number of investigators. Bary ('20) studied Raffo's soluble sulphur (here termed hydrophilic colloidal sulphur), and came to the conclusion that the substance contained not only sulphur but polythionates. He thought the solution was made stable by the presence of small amounts of electrolytes. Freundlich and Scholz ('22) made a very extensive study of the so-called soluble sulphur and concluded that it was largely pentathionic acid. They base their conclusion on the following reactions which would take place if pentathionic acid were present.

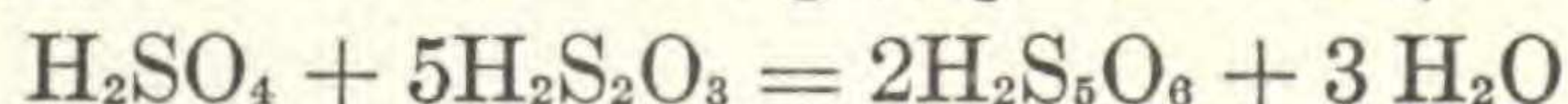


By the aid of this reaction they were able to determine qualitatively and quantitatively the pentathionic acid. The qualitative test was made by the addition of an alkali which precipitated out the sulphur in the form of a white turbid solution. They state that this test applies only to pentathionic acid, and to no other sulphur compound containing oxygen, such as any of the well-known acids or oxides. The quantitative test is made by treating the colloidal solution with normal  $\text{NH}_4\text{OH}$ , forming ammonium thiosulphate and titrating this with 0.01N iodine solution. With hydrophobic colloidal sulphur these tests were negative. They designate colloidal sulphur in this form as  $\text{S}\lambda$  and the form associated with pentathionic acid as  $\text{S}\mu$ . When  $\text{S}\mu$  is precipitated out of hydrophilic colloidal sulphur it probably becomes  $\text{S}\lambda$ . Such a change also takes place when pentathionic acid is treated with  $\text{H}_2\text{S}$ .

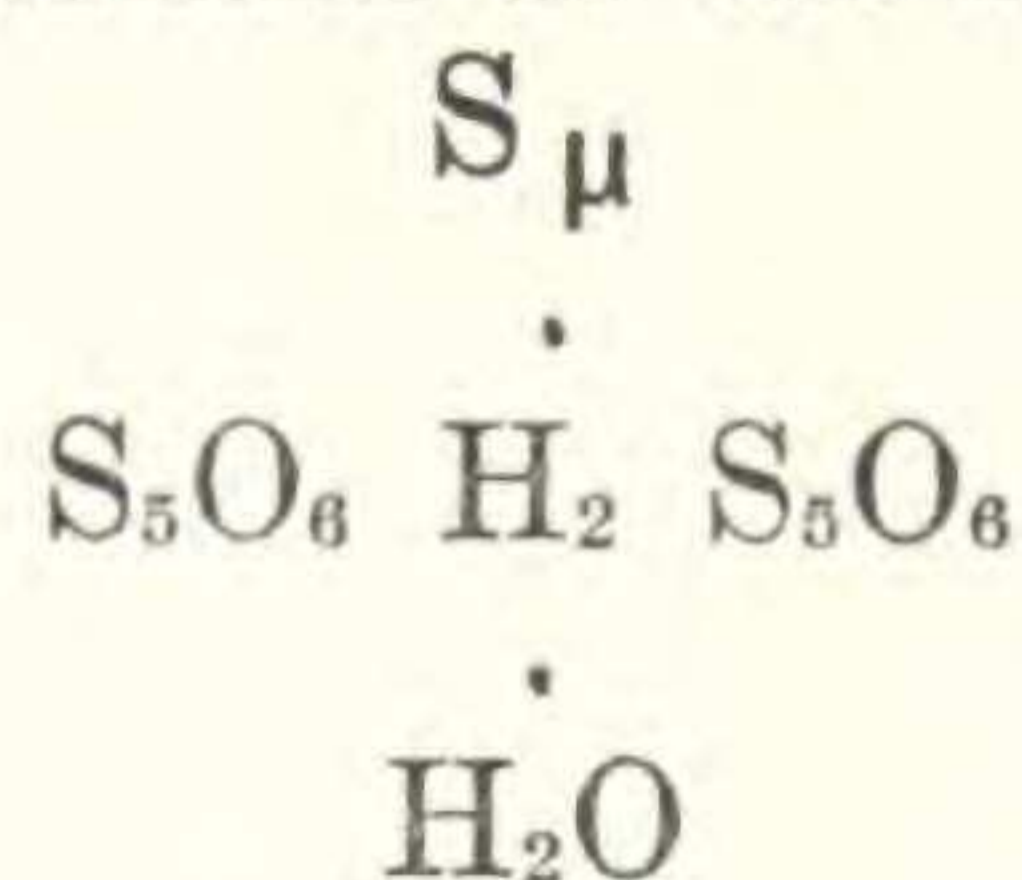
According to these workers, sodium thiosulphate and sulphuric acid react as follows:



By the action of remaining sulphuric acid,



The pentathionic acid then joins with sulphur ( $\text{S}_\mu$ ) and water to form the hydrophilic colloid of the following structure:



The possibility of such a structure is based on the fact that a compound containing so many oxygen ions must necessarily have a great affinity for water. Moreover, a molecule containing so many sulphur atoms would, because of its residual valence, account for its combining with other atoms of sulphur. This being true, pentathionic acid would have the property of combining between molecules of sulphur and water. In other words, it is an adsorptive medium for both these substances. A similar phenomenon is described and illustrated by Langmuir ('17) in his studies of secondary valences in mixtures of fats and water.

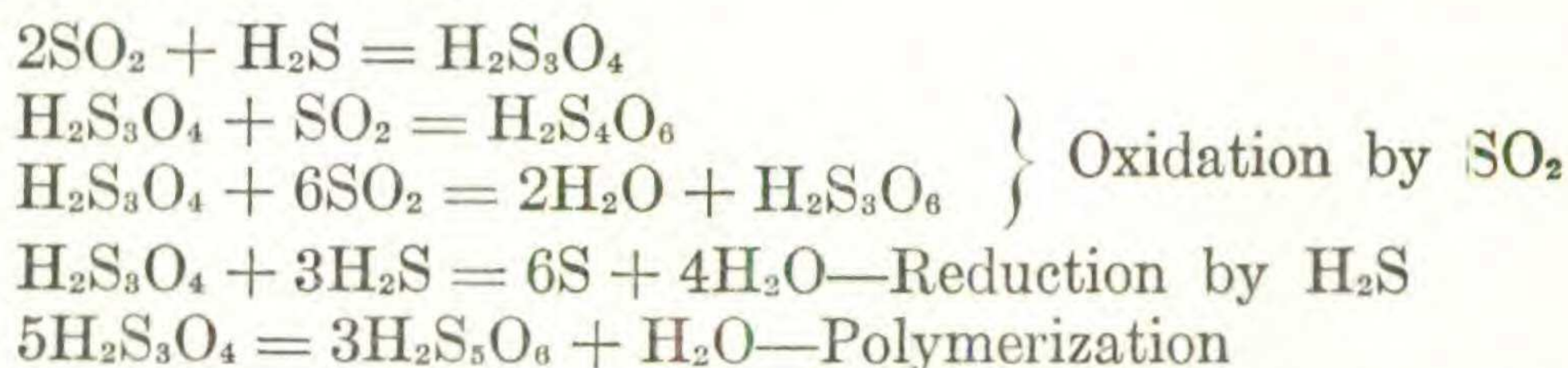
Having no such adsorption medium present in hydrophobic colloidal sulphur, the  $\text{S}_\lambda$  absorbs water and forms the grouping  $\text{S}_\lambda \cdot \text{H}_2\text{O}$ , which is a typical suspension colloid, poorly hydrated and gradually settling out.

The chemical nature of pentathionic acid has been very thoroughly studied. It was discovered by Wackenroder ('46) in 1845. He prepared the acid by passing  $\text{H}_2\text{S}$  into a saturated solution of  $\text{SO}_2$ , always keeping the excess of the latter. By calculations he arrived at the formula of  $\text{H}_2\text{S}_5\text{O}_6$ . For quantitative determinations he precipitated the acid with an alkali, in much the same way as reported by Freundlich and Scholz ('22) for hydrophilic colloidal sulphur. He also found that salts precipitated it.

After the discovery of this acid considerable controversy arose as to its existence in a pure state. Spring ('82) states that it is his opinion that the so-called pentathionic acid consists of a solution of sulphur in tetrathionic acid and that salts obtained from this solution are simply tetrathionates containing admixed sulphur. That this conclusion was partially correct was proved by Shaw ('83). He could produce pure pentathionic acid, but at times such an admixture as obtained by Spring would be obtained. A close relationship undoubtedly exists between pentathionic acid

and sulphur. Shaw prepared his pentathionic acid by passing simultaneously hydrogen sulphide and sulphur dioxide into 3 liters of distilled water for 32 hours, the sulphur dioxide being kept slightly in excess. This controversy was definitely settled by Debus ('88). His work is summed up by Mellor ('17) in the chapter on the compounds of sulphur and oxygen.

The properties of pentathionic acid have been more recently studied by Raschig ('20) and Riesenfeld and Feld ('21). The latter state that the action of  $H_2S$  and  $SO_2$  forms the hypothetical acid  $H_2S_3O_4$  as an intermediary product and that by its oxidation and reduction pentathionic acid is formed; they give the following reactions:



They studied the action of acid and alkali and found that the acid was unstable in both conditions.

It is therefore evident that the hydrophilic colloidal sulphur prepared according to the methods of Selmi ('52), Raffo ('08), Odén ('13), and others, is pentathionic acid. That this is an oxidation product of sulphur seems a logical conclusion. The influence of the hydrogen-ion concentration also points toward pentathionic acid as being the toxic factor in all of the preceding experiments. Flowers of sulphur, hydrophobic colloidal sulphur, and especially hydrophilic colloidal sulphur exhibited toxicity only at  $P_H$  4.2–5.4, because of the fact that pentathionic acid is destroyed when in a solution of higher or lower hydrogen-ion concentration.

To obtain further proof of the toxicity of pentathionic acid the hydrophilic colloidal sulphur was freed of this acid. The colloidal sulphur was prepared by the following method, which is only a slight modification of the one used in previous experiments: Thirty cc. of a saturated solution of sodium thiosulphate were slowly added to 10 cc. of concentrated sulphuric acid. The mixture was warmed and filtered through glass wool. The filtrate was then coagulated with sodium chloride and centrifuged. The coagulum was peptized in 100 cc. of distilled water and again centrifuged to remove insoluble sulphur. Coagulation,



centrifuging, and peptizing were repeated 3 times, and the final coagulum peptized in 100 cc. of distilled water. The reaction of this peptized solution was  $P_H$  4.2. Seventy-five cc. of this solution, for convenience designated No. 1, were treated with 25 cc. of normal ammonium hydroxide and let stand 24 hours, a white precipitate being formed. This was neutralized and centrifuged. The residue was suspended in 75 cc. of distilled water and designated solution 2. The filtrate, No. 3, was again treated with 25 cc. of normal ammonium hydroxide and left for 24 hours. A slight precipitate was formed. This was neutralized, the precipitated sulphur centrifuged out and suspended in 10 cc. of water, and this last designated solution 4; and the filtrate, No. 5. Seventy-five cc. of the filtrate, No. 5, were again treated with 25 cc. of normal ammonia and left for 24 hours. No precipitate formed. This was neutralized and called solution 6.

Fifty cc. of solution 2 were treated with 25 cc. of normal ammonium hydroxide and kept for 24 hours. It was then neutralized and centrifuged. The residue was suspended in 50 cc. of distilled water and designated solution 7. Twenty-five cc. of this solution were treated with 10 cc. of ammonia, allowing the usual interval, then again neutralized, centrifuged, and suspended in 25 cc. of water, constituting solution 8. A portion of each of these solutions was tested for pentathionic acid, with the result that Nos. 3, 5, 6, and 8 gave no positive test; Nos. 1, 2, and 7 gave positive tests, but 2 and 7 only a slight indication.

These solutions were then tested in respect to toxicity in closed-ring cells, using the spores of *Botrytis cinerea* and *Colletotrichum Gossypii*. The cultures were placed at 22° C. for 24 hours, and the results, which are averages of duplicate cultures, are given in table v.

TABLE V  
PERCENTAGE OF GERMINATION

Organism	No. of solution								Ck.
	1	2	3	4	5	6	7	8	
<i>Botrytis cinerea</i>	0	10	50	41	55	55	26	54	53
<i>Colletotrichum Gossypii</i>	0	7	61	54	65	64	18	63	61

The amount of killing was directly proportional to the amount of pentathionic acid present. No. 8 contained as much sulphur as No. 1 but was not toxic.

Another experiment was conducted to ascertain if aerated flowers of sulphur produces pentathionic acid. Two lots of 50 gms. each of flowers of sulphur were placed in wash bottles. The 2 bottles were placed in series each connected with wash bottles containing distilled water to collect any volatile water-soluble compound that might come over. Air was passed through one series and air deprived of oxygen through the other. Aeration was continued for 72 hours. At the end of this time  $H_2S$  was passed into the distilled water wash bottles and permitted to stand for 12 hours. A slight precipitate was noted in the distilled water through which air containing oxygen had passed. The series without oxygen gave no precipitate. This afforded definite proof that pentathionic acid is an oxidation product of flowers of sulphur at ordinary temperatures. A concentrated solution of sodium chloride was added to the aerated sulphur suspensions, and centrifuged; the residue was resuspended in water and again centrifuged. Hydrogen sulphide was then passed into the supernatant liquid. A precipitate developed only in the one in which oxygen was present.

A similar series was arranged using precipitated sulphur containing no pentathionic acid. The distilled water containing the volatile soluble compound and the aerated suspension were tested for pentathionic acid. The former gave a slight precipitate with  $H_2S$  after standing. The suspension gave a much heavier precipitate indicating that the pentathionic acid was adsorbed by the sulphur particle and was not easily driven off by slow aeration. Without oxygen there was no pentathionic acid produced.

The precipitated sulphur was much more easily oxidized than the sublimed flowers of sulphur.

#### PRACTICAL APPLICATIONS

Time has not permitted a more extensive study of this phase of the problem. It was necessary in the first place to determine the compound of sulphur that is toxic to fungi and to develop a material that would act as a fungicide over a sufficient period when sprayed on the plant. The fact that flowers of sulphur must be acted upon by some definite external physical factor has limited its use to only a small section of the country. It has been the aim in this work to develop, if possible, a sulphur compound that would exhibit fungicidal properties regardless of climatic factors and would for that reason have a wide usage over a large

part of the country. To accomplish this the material must yield readily the toxic compound, pentathionic acid. The reaction must be kept slightly acid ( $P_H$  4.0–5.5), as this toxic compound is destroyed above or below this point. It must be readily oxidizable at ordinary temperatures. It should have great adhesiveness; it must not burn the leaves.

Colloidal sulphur has all these properties when tested in the laboratory and greenhouse. It is almost impossible to wash it from the leaves of plants after it has dried. It is difficult to remove it with a strong stream of water. Certainly rain would have little effect upon it.

That colloidal sulphur is readily oxidized has been demonstrated in the foregoing experiments. Kuhl ('21) states that colloidal sulphur bears the same relation to atmospheric oxygen as phosphoric iron, the latter being self-inflammable.

Methods for the preparation of colloidal sulphur mixtures for fungicidal use are being experimented upon. The hydrophilic colloidal sulphur prepared by the method given above is suitable as a spray. It did not burn the leaves of bean, potato, tobacco, rose, and geranium when sprayed on them. By the use of commercial materials this mixture is not too costly for practical purposes. Other methods for its preparation are being tried.

The method for the preparation of hydrophobic colloidal sulphur for trials in the greenhouse was as follows: One gallon of commercial or home-made lime sulphur was diluted with 5 gallons of water. Commercial phosphoric acid was added until the reaction was slightly acid. A milky precipitate of colloidal sulphur was formed. The mixture was allowed to stand a day or two to remove excess  $H_2S$ , and then applied. The advantage of phosphoric acid over other acids is that the calcium acid phosphate formed maintains the proper reaction. This mixture diluted 1:5 with water prevented the germination of *Botrytis cinerea* and *Colletotrichum Gossypii* in aerated cultures. When sprayed on the plant this type of colloidal sulphur does not stick as well as hydrophilic colloidal sulphur but no doubt can be made just as effective a spray by the addition of soluble glue or other suitable spreaders. Any precipitated sulphur to which has been added calcium acid phosphate or another suitable compound for maintaining the slightly acid reaction should be an effective fungicide.

With respect to increasing the value of flowers of sulphur as a spray the writer is not yet prepared to make a definite recom-

mentation. However, the fact that this substance is slowly oxidized at ordinary temperatures leads to the possibility of its being used effectively when treated with compounds that will increase its oxidation. It will also be necessary to add to such a spray an adsorptive material to retain the pentathionic acid as it is produced. Many of the common spreaders now in use may do this. These possibilities are being investigated and will be reported later.

Since the completion of the experimental part of this work there has come to my attention a number of colloidal sulphur preparations that have proved effective as a general spray. Ramsay and Cooke ('22) have prepared a colloidal sulphur that has been used effectively in Australia. They prepare their compound as follows: Ten gallons of home-made lime sulphur (26° Baumé) are diluted with 25 gallons of water in a barrel of 50 gallons capacity. In a suitable vessel 6 pints of strong commercial sulphuric acid are diluted with 9 parts of cold water and allowed to cool. The cold diluted sulphuric acid is then carefully added to the dilute lime sulphur in the barrel, a pint at a time, stirring well until the typical yellow color of the original lime sulphur disappears and until further addition of more acid produces no further precipitation of sulphur. The precipitated sulphur is allowed to settle for a day or two. Three pounds of cheap glue are dissolved in sufficient hot water to render the glue soluble and while still hot is stirred thoroughly into the sulphur. The glue aids in the keeping qualities of the colloidal sulphur. The mixture so prepared is diluted to 250 gallons (with water). This gives a spray containing approximately 5 pounds of precipitated sulphur per 100 gallons.

Thiele ('21) recommends the use of colloidal sulphur in the form of a liquid spray (not dust) for the control of mildews in Germany. He states that it is far more effective than the most finely ground sulphur powder. The colloidal mixtures adhere firmly to the plant and are not blown away by the wind or washed off by rains, as is the powder. Precipitated sulphur as a control for mildew and related fungi has been recommended by Lederle ('22). He prepared this precipitated sulphur as follows: Solution I: 250 gms. of sodium hyposulphite are dissolved in  $\frac{3}{4}$  liter of hot water. Solution II: 250 gms. of sodium bisulphate are dissolved in  $\frac{3}{4}$  liter of hot water. Solution III: 10 gms. of glue are dissolved in  $\frac{1}{4}$  liter of hot water. Solution III is then stirred

while hot into solution I. After diluting solutions I and II each with 4 liters of water they are mixed and let stand for 3–18 hours when the mixture is ready for use. It is somewhat unstable and should be used within a few days, preferably the next morning.

Kuhl ('21) experimented with De Haen's colloidal soluble sulphur<sup>1</sup> and found it to be very effective in controlling mildews and related diseases. He stated that the mixture was very adhesive and that it did not burn the leaves. He believed that the increased effectiveness of this type of sulphur over other sulphur sprays was due to its increased chemical activity.

Barker and Wallace ('22) describe a new method for sulphur fumigation for the greenhouse. In previous studies they found that the fungicidal value of sulphur depended upon its being applied as extremely finely divided particles. Their method is as follows: Air is passed through molten sulphur in a Campbell's "sulphur vaporiser," the temperature of the sulphur being kept just above the melting point and well below the ignition point. The melting point of sulphur is about 115° C. and its ignition point in the air is about 260° C. The most satisfactory temperature is around 170° C. Under these conditions an abundant cloud of sulphur in the particulate condition is produced. An improvement in the yield of particulate sulphur is effected if the current of air is passed into the molten sulphur through a perforated nozzle. By means of an attached delivery tube the particulate sulphur can be discharged in any given direction and on to any definite object. It can be used for general fumigation or for direct spraying.

Another method for fumigation has been described by Vogt ('21), and is as follows: Three-hundred gms. of pure roll sulphur (stick sulphur) contained in a small iron pan is liquefied and brought to the boiling point (448° C.). There is heated at the same time in a circular copper boiler 400 gms. of water. The strongly superheated steam of the latter is forced under high pressure through the boiling sulphur which vaporizes it into small mist-like drops. These drops preserve their liquid form for several hours. They possess a high degree of adhesion not otherwise common to sulphur and do not burn the leaves. A few gms. of sulphur are enough to fill an average greenhouse with clouds of vapor which in a very short time covers all free surfaces.

<sup>1</sup> Manufactured by De Haen at Seelze.

A strong stream of water from the hose did not remove the sulphur from panes of glass. The method is being perfected for open-air use.

### CONCLUSIONS

1. Flowers of sulphur is not sufficiently toxic to inhibit the germination of spores of *Botrytis cinerea*, *Colletotrichum Gossypii*, *Macrosporium sarcinaeforme*, and *Gloeosporium venetum* in closed-ring cells at ordinary temperatures. Spores of *Sclerotinia cinerea* and *Phomopsis Sojae* were inhibited from germination.

2. Finely ground flowers of sulphur was more toxic than the unground flowers under the same conditions but only at a hydrogen-ion concentration of  $P_H$  4.0–5.5.

3. Methods for the preparation of hydrophilic and hydrophobic colloidal sulphur have been devised.

4. Hydrophilic colloidal sulphur was extremely toxic to all the organisms used.

5. Hydrophobic colloidal sulphur was slightly more toxic than the finely ground flowers of sulphur.

6. The chemical and fungicidal properties of lime sulphur were studied. Before application lime sulphur contains 80.7 per cent sulphur as calcium sulphides, 19 per cent as calcium thiosulphate, and .03 per cent as sulphites and sulphates. After exposure to the air for a few hours as a spray the sulphides disappear and increasing amounts of sulphur are formed. The lasting effectiveness of the mixture is due to the precipitated sulphur which is about as toxic as hydrophobic colloidal sulphur.

7. The toxic property of sulphur is not due to  $SO_2$ ,  $SO_3$  or  $H_2S$ , or any of the common acids or oxides of sulphur, or to the sulphur particle. Germ tubes grew normally in a heavy suspension of precipitated sulphur in closed-ring cells.

8. The toxic property of sulphur is only exhibited when oxygen and water are present.

9. By chemical analysis the toxic property of sulphur has been found to be pentathionic acid which is an oxidation compound formed from sulphur and water.

10. Pentathionic acid is volatile and is an active adsorption compound. It is destroyed in acid and alkaline solutions.

11. Finely divided sulphur is more readily oxidized to pentathionic acid at ordinary temperatures than is the flowers of sulphur.

12. Finely divided sulphur has been used as a spray in England, Australia, and Germany, with excellent results.

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